

AMENDMENTS TO THE DRAWINGS:

Fig. 1 has been amended with the legend "Prior Art" to indicate that it is prior art.

REMARKS

I. Introduction

In response to the pending Office Action, Applicant has cancelled claim 25 of the present invention. In addition, Applicant has amended the specification to overcome objections due to minor formalities and amended the drawings to indicate that Fig. 1 is prior art. No new matter has been added.

As claim 25 has been cancelled, Applicant submits that the § 112, first paragraph and § 112 second paragraph rejections of claim 25 are now moot.

For the reasons set forth below, Applicant respectfully submits that all pending claims are patentable over the cited prior art.

II. The Rejection Of Claims 1 And 20-26 Under 35 U.S.C. § 103

Claims 1 and 20-26 are rejected under 35 U.S.C. §103(a) as being unpatentable over Douglas et al. (*Inorg. Chem.*, **2000**, 39, 1828-30) in view of Yang et al. (*Biochem. J.*, **1999**, 338, 615-618). Applicant respectfully traverses this rejection for at least the following reasons.

With regard to the present invention, claim 1 discloses a method for producing a cobalt-protein complex comprising: the step a) of preparing a solution including Co^{2+} ions, a protein, and HEPES, and having a pH of not less than 8.0 and not more than 8.8; and a step b) of adding an oxidizing agent to the solution and thereby making the protein contain particles composed of cobalt.

In the pending Office Action, the Examiner has admitted that Douglas fails to teach or suggest the use of a buffer solution such as HEPES for adjusting the pH. However, the

Examiner alleges that the combination of Yang, which discloses the use of Tris and Good's buffer including HEPES and that buffers catalyze the autooxidation, in view of the disclosure of Douglas, renders claims 1, 20-24 and 26 of the present invention obvious.

Douglas discloses the use of Co^{2+} ions, a protein, (apoferritin), and an oxidizing agent (H_2O_2). Yang discloses using a HEPES buffer agent, Fe^{2+} ions, apoferritin, and an oxidizing agent (oxygen) (see, trace C in Fig. 1 of Yang). Moreover, Yang discloses that Co^{2+} ions may be replaced with Fe^{2+} ions due to the affinity of apoferritin for metal ions (see, page 618, left column, "Discussion," of Yang). However, it appears that from the content of the invention of Yang, trace C in Fig. 1 is illustrated merely to compare with the other examples.

Yang explicitly discloses that iron (II) oxidation is retarded by buffers (see, page 615, right column, lines 5-9). However, in the example shown by trace A, since the pH is not controlled, the protons produced in the formation of $\text{FeO}(\text{OH})$ accumulate, resulting in a pH decrease to 5.2. As a result, it is shown that the oxidation would not proceed without the use of buffers.

In the examples shown by traces B and C, the pH is controlled at pH 7 using the buffers (HEPES) which was once considered to have very low affinity for biologically important metal ions (see, page 618, left column, "Discussion," third paragraph). Trace B shows an example without apoferritin and trace C shows an example with apoferritin, and traces B and C both exhibit oxidation rates higher than trace A.

In the example shown by trace D, the pH is under stat control (pH=7) with no buffer (see, page 616, left column, second paragraph). Trace D exhibits an oxidation rate far higher than traces B and C. Note that the pH stat is controlled by addition of NaOH (see, Douglas, page

1829, right column, second paragraph, and Yang, page 617, left column, lines 14-15 from the bottom).

Based upon the foregoing, traces B and C (under the pH stat control with HEPES buffer) exhibit oxidation rates higher than trace A (without the pH stat control). However, the result from trace D in Fig. 1 implies that when the pH is under stat control, no buffer is preferred. Therefore, Yang suggests that no buffer is preferable so as to improve the oxidation rate.

Furthermore, again the pH is set to pH 7 and accordingly, it is out of the range of 8.0-8.8 recited in claim 1 of the present invention.

For the foregoing reasons, trace C (the example using a HEPES buffer agent, Fe^{2+} ions, apoferritin, and an oxidizing agent) merely illustrates an *undesirable* example for trace D (the example with no buffer). Hence, Yang does not suggest the use of HEPES buffer for a method for producing a cobalt-protein complex as disclosed in the present invention. Rather, Yang appears to teach away from the use of HEPES buffer.

In the case of considering that traces B and C in Fig. 1 of Yang exhibit oxidation rates slower than trace D, the following contradiction arises in using the Co^{2+} ions.

The Tris buffer has a stronger affinity for metal ions as compared with other buffers such as HEPES (see, Yang, page 617, left column, lines 14-16 from the bottom, and page 618, left column, "Discussion," third paragraph). Thus, the skilled person would use the Tris buffer, which is regarded most preferable to retard the oxidation rate in Yang, to control the pH stat.

When the buffers other than the HEPES buffer (e.g., the Tris buffer) are used, no cobalt oxide is formed within proteins (see, page 15, line 20 - page 16, line 9 of the present invention).

The relationship between the Co^{2+} ions and the Tris buffer is such that no Co^{2+} -Tris associated pairs are formed, not that the reaction rate is remarkably slow. It has been considered that the affinity of Tris for Co^{2+} ions in the Co^{2+} -Tris associated pairs is stronger than in the Fe^{2+} -Tris associated pairs, and an oxidizing agent cannot break the couple of Co^{2+} -Tris associated pairs.

Now, the differences between Fe and Co (and Ni), which are the transition metals in Group 8, will be described in view of ligand.

The solution containing Fe ions to which ligands, water molecules are coupled is only brown. On the other hand, the solution containing Co ions to which ligands are coupled is basically pink, but is green in some cases. This means that the ligands (water molecules) have more affinity for Co ions than for Fe ions. Therefore, comparing Fe and Co, both which are the transition metals in Group 8, the affinities for ligands are different from each other. The affinity, of course, depends on kinds of ligands.

The buffers represented by HEPES and Tris are partially coupled to ligands of metal ions. Likewise the above explanation on the water molecules, the affinity of HEPES for Fe ions, the affinity of Tris for Fe ions, the affinity of HEPES for Co ions, and the affinity of Tris for Co ions are all different from one another.

Among these affinities, the affinity of Tris for Co ions is strongest and is not broken by an oxidizing agent. Thus, as described in page 15, line 20 - page 16, line 9 of the present invention, when the buffers other than the HEPES buffer (e.g., the Tris buffer) are used, no cobalt oxide is formed in the protein.

According to Yang, in order to form a cobalt oxide by introducing Co^{2+} ions to the protein, the Tris buffer is most preferable to retard the oxidation rate to control the pH stat (see, Yang, page 617, left column "Discussion," third paragraph). However, in fact, the affinity of Tris for Co ions is stronger than the other three affinities, and thus the affinity of Tris is not broken by an oxidizing agent and no cobalt oxide can be obtained.

Therefore, even if one skilled in the art attempts to form cobalt oxide by introducing Co^{2+} ions to the protein on the basis of the disclosure of Yang, no cobalt oxide can be obtained. Moreover, according to the present invention, no cobalt oxide can be obtained even using the buffers other than HEPES. Thus, it would be difficult for one skilled in the art to use the HEPES buffer so as to form a cobalt oxide by introducing Co^{2+} ions to the protein.

Thus, in view of the aforementioned reasons, the combination of Douglas and Yang does not render claim 1 of the present invention obvious.

More specifically, assume that one skilled in the art attempts to form cobalt oxide by introducing Co^{2+} ions to the protein. If some cobalt oxides can be obtained using Tris or TAPS, it would be easy for one skilled in the art to select HEPES as an appropriate buffer among various buffers. However, in the actual experiments, such selection itself is denied.

According to the inventor's experiments, it was found that when a cobalt oxide was formed by introducing Co^{2+} ions to the protein, buffers other than HEPES did not result in obtaining a cobalt oxide. Therefore, one skilled in the art who conducted experimentation on the basis of the disclosure of Yang would anticipate that HEPES does not allow for obtaining a cobalt oxide.

Even considering the differences between Fe and Co (and Ni), which are the transition metals in Group 8, in view of ligand, one skilled in the art who conducted experimentation on the basis of the disclosure of Yang would anticipate that HEPES does not help in obtaining a cobalt oxide, and thus would further anticipate that any buffer is unnecessary. Furthermore, there would be no reason for the artisan to select HEPES from among various buffers.

The US Patent Application No. 2004-0197884 A, which was published in International Publication WO2004/033366 at the international stage, discloses that not only a buffer but also carbonate ions (or hydrogen carbonate ions) must be added to Ni, which belongs to Group 8, or Cu, which is suggested in Yang. It is assumed that carbonate ions (or hydrogen carbonate ions) are essential since Fe, Co and Ni, all which belong to Group 8, have differences in ligand.

Therefore, Yang suggests Ni and Cu, but U.S. Pat. Appl. No. 2004-0197884 A confirms that even when experiments are conducted in accordance with the disclosure in Yang, Ni or Cu compound cannot be obtained.

Regarding proteins having a hollow other than apoferritin (e.g., Dps protein), Yang discloses that cobalt oxide can be obtained by using HEPES.

Based upon the foregoing, if Yang is regarded as disclosing that the use of buffer makes it possible to slow the oxidation rate as compared with the case of not using the buffer while maintaining a constant pH, no cobalt oxide is formed in the protein even by the experiments in accordance with the disclosure of Yang, and thus a complete contradiction arises.

In order to establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 180 USPQ 580

(CCPA1974). At a minimum, as both Douglas and Yang fail to teach or suggest a method for producing a cobalt-protein complex comprising: the step a) of preparing a solution including Co^{2+} ions, a protein, and HEPES, and having a pH of not less than 8.0 and not more than 8.8; and a step b) of adding an oxidizing agent to the solution and thereby making the protein contain particles composed of cobalt, it is submitted that Douglas and Yang, alone or in combination, do not render claim 1 obvious. Accordingly, it is respectfully requested that the § 103 rejection of claim 1, and any pending claims dependent thereon be withdrawn.

III. All Dependent Claims Are Allowable Because The Independent Claim From Which They Depend Is Allowable

Under Federal Circuit guidelines, a dependent claim is nonobvious if the independent claim upon which it depends is allowable because all the limitations of the independent claim are contained in the dependent claims, *Hartness International Inc. v. Simplimatic Engineering Co.*, 819 F.2d at 1100, 1108 (Fed. Cir. 1987). Accordingly, as claim 1 is patentable for the reasons set forth above, it is respectfully submitted that all pending dependent claims are also in condition for allowance.

IV. Conclusion

Having fully responded to all matters raised in the Office Action, Applicant submits that all claims are in condition for allowance, an indication of which is respectfully solicited.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper,

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including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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